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Attachment A

FACSIMILE TRANSMITTAL FORM

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FROM: Dr. Lentz

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MESSAGE:

M. Rigdon Lentz, M.D.
397 Wallace Road, Suite 314
Nashville, TN 37211

1165 3/3

to: Patrea Pabst
Arnall Golden& Gregory, LLP
2800 one Atlantic Center
1201 West Peachtree street
Atlanta, Georgia 30309-3450

subj: Patent - Method and Compositions for Treatment of Cancers

Dear Mrs Pabst:

Thank you for your through work and excellent job in preparing this application. I have reviewed it as you requested and have offered some few remarks of a technical nature and have tried to add the additional information where indicated. These additions are as follows:

1. pg.1, line.19. after GM-CSF, "erythropoetin, thrombopoetin, G_CSF,M-CSF and SCF."

2. pg.2, line.27 after ultrafilter "or parallel plate filter" line.29 after filter "at least"

3. pg 3, line. 6 after IL-2,"IFNs". line. 7 after increases "the inflammation against tumors by allowing cytokines, such as TNF to work effectively."

4. pg. 3, line. 10 after taxol" and other drugs which may be synergistic in effect with "unblocked" cytokines." line. 19. after receptors "or inhibitors to IL-2, IL-6, gamma interferon,or other pro-inflammatory signaling as well as white cell activation."

5. pg. 3 line.27 after polypropylene"ethylene polyvinyl alcohol or polysulfone"

6. pg. 4 line.9 add sentence after components "Specific absorbing columns can also be employed to selectively remove specific cytokine and cellular inhibitors from the filtered plasma so that the so treated ultrafiltrate of plasma maybe returned to the patient to achieve the desired effect."

7. pg. 4 line 30 Kuraray Co., Ltd
1-12-39, Umeda, Kite-ku,
Osaka 530 Japan

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8. pg. 5, line10 change "weight" to "volume". line13 change "a compatible plasma" to "normal saline"

9. pg.7, line 25 after size "or which suggests tumor inflammation. " line. 27 after tumors "and/or inflammation".

10. pg.8 line. 10 "glioblastomas" line15 "TNF alpha and beta receptors"

11. pg.9 line 23 C **Cytokines** The biologic activity and clinical effectiveness of pro-inflammatory cytokines is augmented in the patient with cancer and other states of acquired immune tolerance by ultrapheresis. Specifically TNF both TNF alpha and TNF beta, in doses 100 to 500 micgms per meter squared body surface area (M2 BSA). Monocyte and lymphocyte activation by INFs -alpha, beta and gamma is augmented. The IL-1 and IL-2 receptor antagonists are removed by this form of ultrapheresis thereby upregulating the in vivo activity of these cytokines. An 80kd glycoprotein has been recently found, full description pending, which is responsible for inhibiting blastiod transformation in advanced malignancy, chronic infectious disease and pregnancy and appears to be responsible for the loss of delayed hypersensitivity reactions in these diseases and it too is remove by this process. This is significant because in removing this type of suppression, vaccines of all types will work better. Dosage regimes for IFN -α&β 3 M units sq three times a week up to 20m units /M2 BSA daily. IFN-γ 100 to 1000 micgms per day.

pg. 9, line28 change "TNA-alpha" to "TNF R-1&R-2 receptor/inhibitor molecules"

11. pg.10, line.14 Tamoxifen plays a role in not only blocking of estrogen receptors but also certain growth factor receptors such as EDGF,FDGF, TDGF-B,and PDGF and therefore may be complimentary to inflammation against cancers provoked by ultrapheresis.

12. pg. 10 line.15 **Radiation** Because radiation therapy is so destructive of normal tissue, causing tumors to die partially by an inflammatory attack allows the use of lower doses of radiation to kill residual tumor cells and spare normal tissue. It is anticipated that by using this form of immunotherapy as initial therapy, that subsequent effective doses of radiation could be reduced in half. It is also well established that TNF kills tumor cells by generating free oxygen radicals, hydroxal radicals and halide ions, radiation therapy generates carbonium ions in tissue and the combination of the two is more effective in kill cancer cells than either alone.

13. pg.12 line.15. change "TNF-alpha" to "TNF-R-1&R-2 receptors"

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I look foreward to discussing these changes with you. I am certain that you will do far better than I at making all this clear. Thank you again for all your good work.

Sincerely

M Rigdon Lentz

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Fax (615) 834-8004

APPLICATION

FOR

UNITED STATES LETTERS PATENT

BY

M. RIGDON LENTZ

FOR

METHOD AND COMPOSITIONS FOR TREATMENT OF CANCERS

540515

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METHOD AND COMPOSITIONS FOR TREATMENT OF CANCERS

Abstract

A method to treat cancer uses ultrapheresis, refined to remove compounds of less than 120,000 daltons molecular weight, followed by administration of replacement fluid, to stimulate the patient's immune system to attack solid tumors. In the preferred embodiment, the patient is ultrapheresed using a capillary tube ultrafilter having a pore size of 0.02 to 0.05 microns, with a molecular weight cutoff of 120,000 daltons, sufficient to filter one blood volume. The preferred replacement fluid is ultrapheresed normal plasma. The patient is preferably treated daily for three weeks, diagnostic tests conducted to verify that there has been shrinkage of the tumors, then the treatment regime is repeated. The treatment is preferably combined with an alternative therapy, for example, treatment with an anti-angiogenic compound, one or more cytokines such as TNF, gamma interferon, or IL-2, or a procoagulant compound. The treatment increases endogenous, local levels of cytokines, such as TNF. This provides a basis for an improved effect when combined with any treatment that enhances cytokine activity against the tumors, for example, treatments using alkylating agents, doxyrubicin, carboplatinum, cisplatinum, and taxol. Alternatively, the ultrapheresis treatment can be combined with local chemotherapy, systemic chemotherapy, and/or radiation.

METHOD AND COMPOSITIONS FOR TREATMENT OF CANCERS

Background of the Invention

The present invention is generally in the field of enhancing an immune response, and particularly relates to the removal of inhibitors of immune mediators, in combination with anti-angiogenic compounds, cytokines, compounds inducing a procoagulant state, chemotherapeutics and/or radiation.

Conventional cancer therapy is based on the use of drugs and/or radiation which kills replicating cells, hopefully faster than the agents kill the patient's normal cells. Surgery is used to reduce tumor bulk, but has little impact once the cancer has metastasized. Radiation is effective only in a localized area.

The treatments can in themselves kill the patient, in the absence of maintainance therapy. For example, for some types of cancer, bone marrow transplants have been used to maintain the patient following treatment with otherwise fatal amounts of chemotherapy. Efficacy has not been proven for treatment of solid tumors, however. "Cocktails" of different chemotherapeutic agents and combinations of very high doses of chemotherapy with restorative agents, for example, GM-CSF, to restore plateler and white cell levels, are used to treat aggressive cancers.

Other treatments have been tried in an attempt to improve mortality and morbidity. Vaccines to stimulate the patient's immune system have been attempted, but not with great success. Various cytokines, alone or in combination, such as tumor necrosis factor, interferon gamma, and IL-2 have been used to kill cancers, but have not produced cures. More recently, anti-angiogenic compounds such as thalidomide have been tried in compassionate use cases and shown to cause tumor remission. In animal studies,

compounds inducing a procoagulant state, such as an inhibitor of protein C, have been used to cause tumor remission. New studies have shown that cytokine receptors, such as tumor necrosis factor receptors (TNF-Rs) are released in a soluble form from tumor cells, in high concentrations relative to normal cells, which may block the immune system's attack on the tumor cells (Jablonska and Petruska, Arch. Immunol. Ther. Exp. (Warsz) 1997, 45(5-6), 449-453; Chen, et al., J. Neuropathol. Exp. Neurol. 1997, 56(5), 541-550).

U.S. Patent No. 4,708,713 to Lentz describes an alternative method for treating cancer, involving ultrapheresis to remove compounds based on molecular weight, which promotes an immune attack on the tumors by the patient's own white cells. Although results have been extremely promising, the treatment usually only produces remissions, not cures.

Despite all of these efforts, many patients die from cancer; others are terribly mutilated. It is unlikely that any one therapy will be effective to cure all types of cancer.

It is therefore an object of the present invention to provide a method and compositions for treatment of solid tumors.

It is a further object of the present invention to provide a method and compositions that does not involve non-selective, extremely toxic, systemic chemotherapy.

Summary of the Invention

A method to treat cancer uses ultrapheresis, refined to remove compounds of less than 120,000 daltons molecular weight, followed by administration of replacement fluid, to stimulate the patient's immune system to attack solid tumors. In the preferred embodiment, the patient is ultrapheresed using a capillary tube ultrafilter having a pore size of 0.02 to 0.05 microns, with a molecular weight cutoff of 120,000 daltons, sufficient to filter one blood volume. The preferred replacement fluid is ultrapheresed

normal plasma. The patient is preferably treated daily for three weeks, diagnostic tests conducted to verify that there has been shrinkage of the tumors, then the treatment regime is repeated.

The treatment is preferably combined with an alternative therapy, for example, treatment with an anti-angiogenic compound, one or more cytokines such as TNF, gamma interferon, or IL-2, or a procoagulant compound. The treatment increases endogenous cytokines, such as TNF. This provides a basis for an improved effect when combined with any treatment that enhances cytokine activity against the tumors, for example, treatments using alkylating agents, doxyrubicin, carboplatinum, cisplatinum, and taxol. Alternatively, the ultrapheresis treatment can be combined with local chemotherapy, systemic chemotherapy, and/or radiation.

Brief Description of the Drawings

Figures 1 and 2 are schematics of the system for ultrapheresis.

Detailed Description of the Invention

The methods and devices disclosed herein are useful for treatment of patients with cancer, immune-mediated disorders, chronic parasitism, some viral diseases, and other disorders characterized by elevated levels of TNF receptors. Examples demonstrate efficacy in treating cancer patients.

I. Ultrapheresis

A. Ultrapheresis System

1. Filters

The filter must be biocompatible, and suitable for contact with blood, without causing excessive activation of platelets or clotting. Such devices for use in kidney dialysis are well known. For use herein, the filter membranes, which will typically be a biocompatible or inert thermoplastic such as polycarbonate or polypropylene, having a pore size of between 0.02 and 0.05 microns. The actual pore size that yields the desired cutoff of approximately 120,000 daltons is determined based on the fluid flow geometry, shear

forces, flow rates, and surface area. The effective cutoff for a capillary membrane filter with a pore size of 0.09 microns is 120,000 daltons, with a serving coefficient of between 10 and 30%. This results in only a trivial amount of IgG being removed from the patient's blood. The filter membrane should be less than about 25 microns, preferably less than about 10 microns, thick. Suitable materials for the ultrafilter membrane include sheets of polytetrafluorethylene (Teflon R) and polycarbonate resins. The permeable membrane should not cause blood clotting or otherwise react with the blood.

Devices will typically be either parallel plate filters or capillary membrane filters. These can be adapted from devices currently in use for kidney dialysis. The capillary membrane filters will typically have a surface area of between about 0.25 and 1 m^2 for use with children and between about 1 and 3 m^2 for use with adults. The parallel plate filters will typically have a surface area in the range from 0.1 and 2 cm^2/ml of blood to be filtered.

Staged filters can also be used, which have different pore sizes and/or geometries or surfaces areas, to provide for a "staggered" removal of materials from the blood. Alternatively, although not at this time preferred, one can use differential centrifugation, to provide for an appropriate separation of blood components [illegible].

A preferred membrane is one in which the pores are made by electron beams directed perpendicularly to the surface because the size and density of the pores can be accurately controlled in this manner. The pores are essentially circular in cross section so the effective pore size is the actual pore size. The effective pore size of ultrafiltered media having pores with non-circular cross sections shall be the diameter of a circular pore which will pass molecules or other components of an equivalent size to the molecules or other components which pass through the filter medium in question.

Suitable devices can be obtained from Asahi Chemical Company and Kuraray, Japan. [check names and provide addresses]

KURARAY Co., LTD
1-12-39, Umeda, Kita-ku,
Osaka 530 Japan.

2. Process Controls and Fluid Handling

The patient will typically be connected to the blood processing device using standard intravenous tubing, with connections similar to those used for plasmapheresis, so that blood can be removed from the patient at one site and returned at another. The tubing is connected to a pump that controls the flow rate so that in the preferred embodiment one blood volume (based on approximately 7% of the total body weight) is processed over a period of approximately 2 1/2 hours. The filtrate is then returned from the filtration device to the patient at the second site. Standard microprocessor controls can be used to regulate the blood flow, for example, by monitoring the weight of the blood products being removed, in combination with flow rate monitors and pump speed.

The entire system should be first flushed with a ~~compatible plasma~~ saline and then treated with an anticoagulant or anticlotting agent, such as sodium heparin, to be sure that there are no locations within the system where blood clotting can occur. Moreover, small amounts of anticoagulants should be continually introduced into the blood stream directed to the ultrafilter to ensure that no clotting occurs during the filtration process. All of the surfaces of the system which come in contact with the blood and fluids which are infused into the patient must be sterilized prior to commencing treatment.

Figure 1 illustrates a system for ultrapheresis. Blood is removed from a patient by means of a venous catheter 10 with the distal lead 11 thereof disposed in the superior vena cava 12 leading to the patient's heart 13. The blood passes through conduit 14 to a drip chamber 15 and then into pump 16 which controls the pressure of the blood to the separation unit 17, preferably an ultrafilter as shown, through conduit 18. A pressure gauge 19 is provided on conduit 14 to continually monitor arterial pressure. A syringe pump 20 feeds an anti-clotting drug such as sodium heparin to conduit 18 to prevent

the clotting of blood in the ultrafilter 17. In the ultrafilter 17 the blood stream passes over the ultrafilter medium or membrane 21 under pressure. The blood fraction including the low molecular weight components passes through the membrane 21 and is discharged as permeate through conduit 22. The retentate or treated blood containing the high molecular weight components, which include whole blood cells and platelets, is discharged into conduit 23 which ultimately leads back to the patient. Volumetric pump 27 passes a controlled amount of permeate to a container 28 for containment and for measuring. Volumetric pump 30, which is preferably the same type and capacity as pump 27, pumps replacement fluid from a container 31 to conduit 24, which directs the fluid to conduit 23 where it mixes with the retentate or treated blood. The treated blood and other components are returned to the patient through venous catheter 34, the distal or discharge end of which is disposed in the brachiocephalic vein. The volumetric pumps 27 and 30 are preferably set either to pump the same total amount of fluid or to pump at the same rate, so that the same volume of fluid which is removed from the patient's blood stream as permeate is returned as replacement fluid. The blood stream in conduit 23 is passed through filter 36 to remove clots or other debris from the blood stream. A drip chamber 37 ensures that no significant quantities of air enter the patient's blood stream. A pressure gauge 38 is provided to continually monitor venous blood pressure.

Figure 2 illustrates another embodiment wherein blood removed from a patient is first passed through conduit 30 to a first ultrafilter 31 to selectively separate a blood fraction with components having molecular weights less than about 1,000,000 Daltons. The retentate from this ultrafiltration which contains the high molecular weight components is returned through conduit 32 to the patient. The permeate from the first ultrafilter 30 is passed through conduit 33 to a second ultrafilter 34 where a

blood fraction having a molecular weight below 30,000 is removed from the permeate stream from the first ultrafilter 30. The permeate from the second ultrafilter 34, which contains the very low molecular weight components such as salts and nutrients may be returned to the patient through conduit 38. The retentate from the second ultrafilter which contains desirable high molecular weight immunoglobulins and other components is discharged through conduit 36 and 13.

Blood should be pumped through the ultrafilter unit at sufficient pressure to cause the blood components having the immunosuppressive effects to pass through the filter but at a velocity which will not excessively shear or otherwise damage the blood cells passing over the membrane. Generally it has been found that the ratio of the area of the membrane to the amount of blood treated per hour should be within about 0.1 to 2 cm/mL. Differential pressure across the membrane should range from about 2 to 20 mM Hg.

3. Replacement Fluids

The patient must receive replacement fluids following filtration. The preferred replacement fluid is ultrapheresis normal plasma, for example, expired plasma obtained from the Red Cross, which has been filtered using the same filter as used to treat the patient. Alternatively, the patient can be administered normal albumin, or fresh frozen plasma diluted with saline.

II. Treatment with Adjuvant Therapies

Standard ultrapheresis is conducted over a period of time until a positive indication is observed. This is typically based on diagnostic tests which show that there has been some reduction in tumor size. The patient is preferably treated daily for three weeks. diagnostic tests conducted to verify that there has been shrinkage of the tumors, then the treatment regime is repeated.

Surgical (or vacuum) removal of necrotic material may be required prior to or during treatment to avoid toxicity associated with high tumor burden.

This procedure has been demonstrated to cause remission of solid tumors in approximately 50% of patients who have failed all other treatment modalities [EXPAND HERE - WHAT KINDS OF CANCERS, WHAT WAS TREATMENT REGIME, WHAT DO YOU MEAN WHEN YOU SAY TREATMENT SHOWED EFFICACY] Types of tumors that are particularly sensitive to the ultrapheresis include epithelial tumors, sarcomas, melanomas, and glioblastomas.

However, it would clearly be advantageous to cause complete remissions. Based on the presumed mechanism that the process is removing immune inhibitors produced by the tumors, especially inhibitors of cytokines and other immune mediators, it is possible to treat the patients with adjuvant or combination therapies, that enhance the results achieved with the ultrapheresis. TNF-alpha receptors are thought to be particularly important immune inhibitors. Therefore, compounds which enhance TNF activity are particularly preferred. These include anti-angiogenic compounds, such as thalidomide, procoagulant compounds, cytokines and other immunostimulants. Standard chemotherapeutic agents and/or radiation can also be used with the ultrapheresis

A. Anti Angiogenic Compounds

Any anti-angiogenic compound can be used Exemplary anti-angiogenic compounds include TNP-470, U. S. patent No. 5,290,807; Angiostatin, U.S. Patent No. 5,639,725; Endostatin, and Thalidomide. Thalidomide is administered once daily, 200 mg orally.

B. Procoagulant Compounds

Protein C is a vitamin K-dependent plasma protein zymogen to a serine protease. Upon activation it becomes a potent anticoagulant.

Activated protein C acts through the specific proteolysis of the procoagulant cofactors, factor VIIIa and factor Va. This activity requires the presence of another vitamin K-dependent protein, protein S, calcium and a phospholipid (presumably cellular) surface. As described in Hemostasis and Thrombosis: Basic Principles and Clinical Practice, 2nd Ed., Colman, R.W., et al., p. 263 (J.B.Lippincott, Philadelphia, PA 1987), protein C circulates in a two-chain form, with the larger, heavy chain bound to the smaller light chain through a single disulfide link. Protein C is activated to activated protein C (APC). Thrombin is capable of activating protein C by the specific cleavage of the Arg^{12}-Leu^{13} bond in the heavy chain. *In vivo*, in the presence of physiological concentrations of calcium, the rate of this activation is enhanced dramatically when thrombin is bound to the endothelial cell cofactor, thrombomodulin. Matschiner, et al., Current Advances in Vitamin K Research, pp. 135-140, John W. Suttie, ed. (Elsevier Science Publishing Co., Inc. 1988) have further reviewed the role of the Vitamin K dependent proteins in coagulation.

Blockage of the natural anticoagulant pathways, in particular the protein C pathway, uses the natural procoagulant properties of the tumor to target the tumor capillaries for microvascular thrombosis, leading to hemorrhagic necrosis of the tumor, as described in U.S. Patent No. 5,147,638 to Esmon, et al. Examples of such compounds include anti-protein C and anti-protein S.

C. Cytokines

[PLEASE PROVIDE DISCUSSION REGARDING WHAT CYTOKINES MIGHT BE USEFUL, DOSAGES AND TREATMENT REGIMES]

D. Anti-TNA receptor molecules.

It is well established that TNF-alpha receptor molecules are shed by tumor cells, and that these molecules appear to inhibit immune mediated

attack by the host on the tumor cells. The ultrapheresis is believed to remove the majority of these soluble receptors. Additional, and/or selective, removal of these molecules can be obtained using antibody, or antibody fragments (single chain, recombinant, or humanized), immunoreactive against the receptor molecules. In the preferred embodiment, these antibodies are immobilized on the ultrapheresis membrane filters, using standard antibody coupling techniques. In the most preferred embodiment, the antibody is reactive with the carboxy-terminus of the shed receptor molecules, thereby avoid concerns with signal transduction by the receptor is still present on the cell surface.

D. Chemotherapeutic Agents

Preferred chemotherapeutic agents are those which are synergistic with TNF, for example, alkylating agents, doxyrubicin, carboplatinum, cisplatinum, and taxol tomoxifen?

E. Radiation

DOSAGES and TREATMENT REGIMES?

III. Examples

Dr. Lentz - please insert examples.

Modifications and variations of the method and compositions described herein will be obvious to those skilled in the art. Such modifications and variations are intended to come within the scope of the appended claims.

I claim:

1. A method for inducing an immune response against transformed, infected or diseased tissue comprising

removing components present in the blood having a molecular weight of 120,000 daltons or less, until the transformed, infected, or diseased tissue is reduced in amount.

2 The method of claim 1 wherein the tissue is a solid tumor.

3. The method of claim 1 wherein the components are removed from one blood volume.

4. The method of claim 1 wherein the components are removed in multiple treatments

5. The method of claim 1 further comprising treating the tissue with an agent selected from the group consisting of anti-angiogenic compounds, procoagulant compounds, cytokines, chemotherapeutic agents, and radiation.

6. The method of claim 1 further comprising selectively removing soluble TNF-alpha receptor molecules.

7. A system for inducing an immune response against transformed, infected or diseased tissue comprising

a device for removing components present in the blood having a molecular weight of 120,000 daltons or less, and

an agent selected from the group consisting of anti-angiogenic compounds, procoagulant compounds, cytokines, chemotherapeutic agents, and radiation.

8. The system of claim 7 wherein the agent is an anti-angiogenic compound.

9. The system of claim 7 wherein the agent is a procoagulant compound.

10. The system of claim 7 wherein the agent is a cytokine.

11 The system of claim 10 wherein the cytokine is selected from the group consisting of....

12. The system of claim 7 wherein the agent is a chemotherapeutic agent.

13. The system of claim 12 wherein the agent is selected from the group consisting of alkylating agents, doxyrubicin, carboplatinum, cisplatinum, and taxol.

14. The system of claim 7 wherein the device includes means for administering radiation to the tissue.

6. The method of claim 1 further comprising selectively removing soluble TNF-alpha receptor molecules.

21-24.

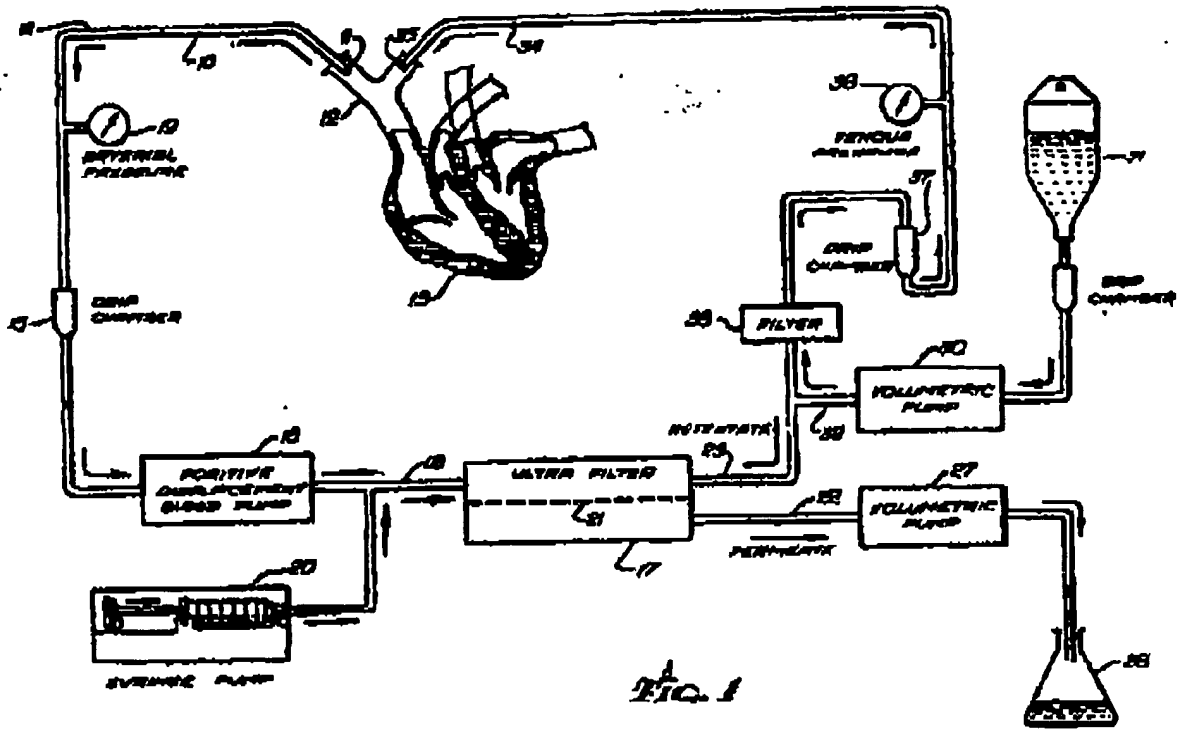

Fig. 1

Fig. 2